

## **Supplemental Material to:**

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**BET bromodomain proteins are required for glioblastoma cell proliferation**

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article/27906](http://www.landesbioscience.com/journals/epigenetics/article/27906)**

**Supplemental File 1. IC<sub>50</sub> values for I-BET151 in CellTiter-Glo measurements in U87MG cells, A172, SW1783, patient-derived GBM cell line (UM20), and GBM stem cells (Glio3).** I-BET151 is more potent than all compounds tested on U87MG GBM cells with the exception of MLN8237 for which it is equipotent. I-BET151 has IC<sub>50</sub> values 30 to 200 fold lower than TMZ in patient-derived GBM cell line and GBM cells, respectively. BKM-120 is a PI3K inhibitor, GDC-0941 is a PI3K inhibitor, PD0332991 is a cyclin-dependent-kinase inhibitor. MLN8237 is an Aurora-kinase inhibitor; MK-2206 is an AKT inhibitor. Data are from one representative experiment performed in triplicate (mean ± SEM) .

**Supplemental File 2. Patient Derived GBM stem cells are Nestin and CD133 positive.** GBM stem cells were cultured as neurospheres and tested for Nestin and CD133 immunoreactivity. A. Western blot showing Nestin, Notch, Musachi, and Actin levels in Glio1 and Glio3 Stem cells. B. CD133 (red), Musahi (red), Nestin (green) and Notch (red) staining for Glio1 and Glio3 GBM stem cells. Dapi was used to label the nuclei (blue).

**Supplemental File 3. Statistics for FUCCI analysis of U87MG cells treated with DMSO or I-BET151.**

**Supplemental File 4. Weights of mice injected with saline or I-BET151.** Two-way ANOVA analysis indicated that there was no statistically significant difference between the I-BET151 and saline treated animals. I-BET151 injected animals weighed 22.8 $\pm$ 0.96g, 24.2 $\pm$ 0.88g, and 25.1 $\pm$ 0.89g at 0 day, 14 day, and 21 day, respectively. Saline injected animals weighed 23.9 $\pm$ 0.76g, 25.6 $\pm$ 1.00g, and 26.5 $\pm$ 0.89g at 0 day, 14 day, and 21 day, respectively.

**Supplemental File 5. I-BET151 Treatment of U87MG cells does not reduce *C-MYC*, *BCL2*, *CDK6*, and *CDK4* levels.** **A. Normalized RNA levels of *C-MYC*, *BCL2*, *CDK4*, and *CDK6* after 24 hours of I-BET151 treatment of U87MG cells.** U87MG cells were treated with I-BET151 500 nM and DMSO for 24 hours. RNA was extracted and the expression of *C-MYC*, *BCL2*, *CDK6* and *CDK4* measured by quantitative RT-PCR. The *Cyclophilin* housekeeping gene was used to normalize the data. Results of one representative experiment shown performed in quadruplicate. **B. Heatmap of Common upregulated or downregulated RNAs in A172 and U87MG cells.** A172 and U87MG were treated for 24 hours with 500nM I-BET and DMSO. RNA was extracted and hybridized to the Affymetrix Array Gene ST. Common genes that were significantly upregulated (red) or downregulated (green) after I-BET151 treatment were then clustered.

**Supplemental File 6. Array results of Glioblastoma (GBM) Stem Cells treated with I-BET151 (500nM) for 24 hours.** RNA was extracted from the indicated Glioblastoma (GBM) stem cells, cDNA synthesized, and hybridized to Affymetrix arrays as described in Materials and Methods. Common upregulated (red) or downregulated (green) genes are shown.

**Supplemental File 7. I-BET151 treatment reduces proliferation of patient derived xenograft cells (PDX).** **A. BRD4 protein levels in a panel of PDX cells.** The indicated GBM lines were assayed for Brd4 expression using anti-Brd4 Western Blot analysis. Actin was used as loading control for the Western blot. **B. I-BET151 affects GBM6 and GBM10 PDX cell line proliferation.** GBM6 and GBM10 were treated with 1 $\mu$ M I-BET151 for 48 hours and the extent of proliferation measured via an EdU incorporation assay. Pictures of the different treatments were taken with 20X magnification objective in the UV and red spectrum and merged utilizing Adobe Photoshop (Blue represents nuclei-Hoechst and green represents EdU-Alexa488 positive cells). **C. Percentage of EdU positive cells in B.** The percentage of EdU positive cells was calculated with the Cellomix Array VTI Scan (Thermo Scientific) and graphed as percentage of DMSO.

**Supplemental File 8. A. BRD2, BRD3 and BRD4 knockdown efficiency.** The efficiency of *BRD2*, *BRD3* and *BRD4* knockdown used in (B) was tested by qRT-



PCR. After *BRD4* knockdown the expression of *BRD2*, *BRD3* and *BRD4* was measured by qRT-PCR and data normalized to *Actin*. Error bars are representative of 3 independent experiments. **B. *BRD2* or *BRD3* knockdown does not affect U87MG proliferation.** U87MG cells were transfected with siRNA against *BRD2*, *BRD3*, *BRD4* and 5 days after the transfection cells were used for an EdU incorporation assay. Pictures of the different treatments were taken with 20X magnification objective in the UV and red spectrum and merged utilizing Adobe Photoshop (Blue represents nuclei-Hoechst and pink represents EdU-Alexa488 positive cells). **C. Percentage of EdU positive cells in B.** The percentage of EdU positive cells was calculated with the Cellomix Array VTI Scan (Thermo Scientific) and graphed as percentage of DMSO.

**Supplemental File 9. A. *CDKN1A* (*p21<sup>Cip1</sup>*) is increased in cells treated with I-BET151.** U87MG and Patient derived GBM10 cells were treated with 500nM I-BET151 for 2h. RNA was extracted and mRNA level of *CDKN1A* were measured by qRT-PCR. Error bars represent the standard deviation. **B. *p21* increases with I-BET151 treatment.** U87MG cells were plated and treated with 500nM and 1000nM concentrations of I-BET151. Cells were harvested and homogenized at 24 and 48 hour time points. Extracts were then prepared using lysis buffer (50 mM Tris, 150 mM NaCl, 1 % Triton X-100, 1X Protease Inhibitor Cocktail, 1mM Microcystin LR). Cells were lysed by the freeze-thaw method (liquid nitrogen/37°C water bath) and further sonicated. The soluble fraction was recovered by centrifugation at 14,000 RPM for 20 min at 4°C. Protein

concentration was measured with the BCA Protein Assay kit (Pierce) and 30 µg of protein from each sample was resolved by SDS-PAGE. The resolved bands were transferred onto a nitrocellulose membrane by Western blotting and then probed with relevant antibodies. Primary Antibodies: anti-p21 antibody (12D1) from Cell Signaling (Cat # 2947S), anti-actin antibody from Cell Signaling (Cat # 8456S), Secondary antibodies: anti-rabbit IgG-HRP antibody from GE Healthcare (Cat # NA9340V).

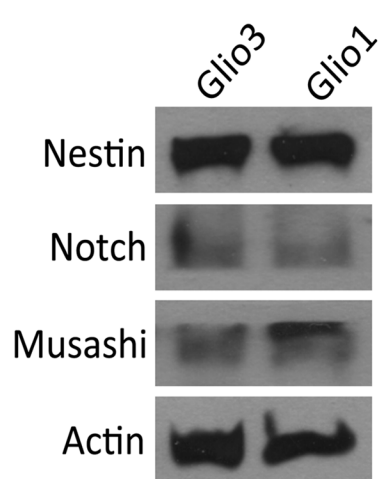
**C. *BCL2* is decreased in GBM cells treated with I-BET151.** U87MG and GBM6 were treated with 500nM I-BET151 for 72 hours. RNA was extracted and mRNA level of *BCL2* measured by qRT-PCR. Error bars represent the standard deviation.

**D. *Hexim1* is downregulated in U87MG cells treated with I-BET151.** U87MG cells were treated with 500nM I-BET151 or DMSO for 24 hours. RNA was extracted and mRNA level of *Hexim1* was measured by qRT-PCR and normalized to the housekeeping gene  $\beta$ -actin. Error bars represent the standard deviation of three independent experiments.

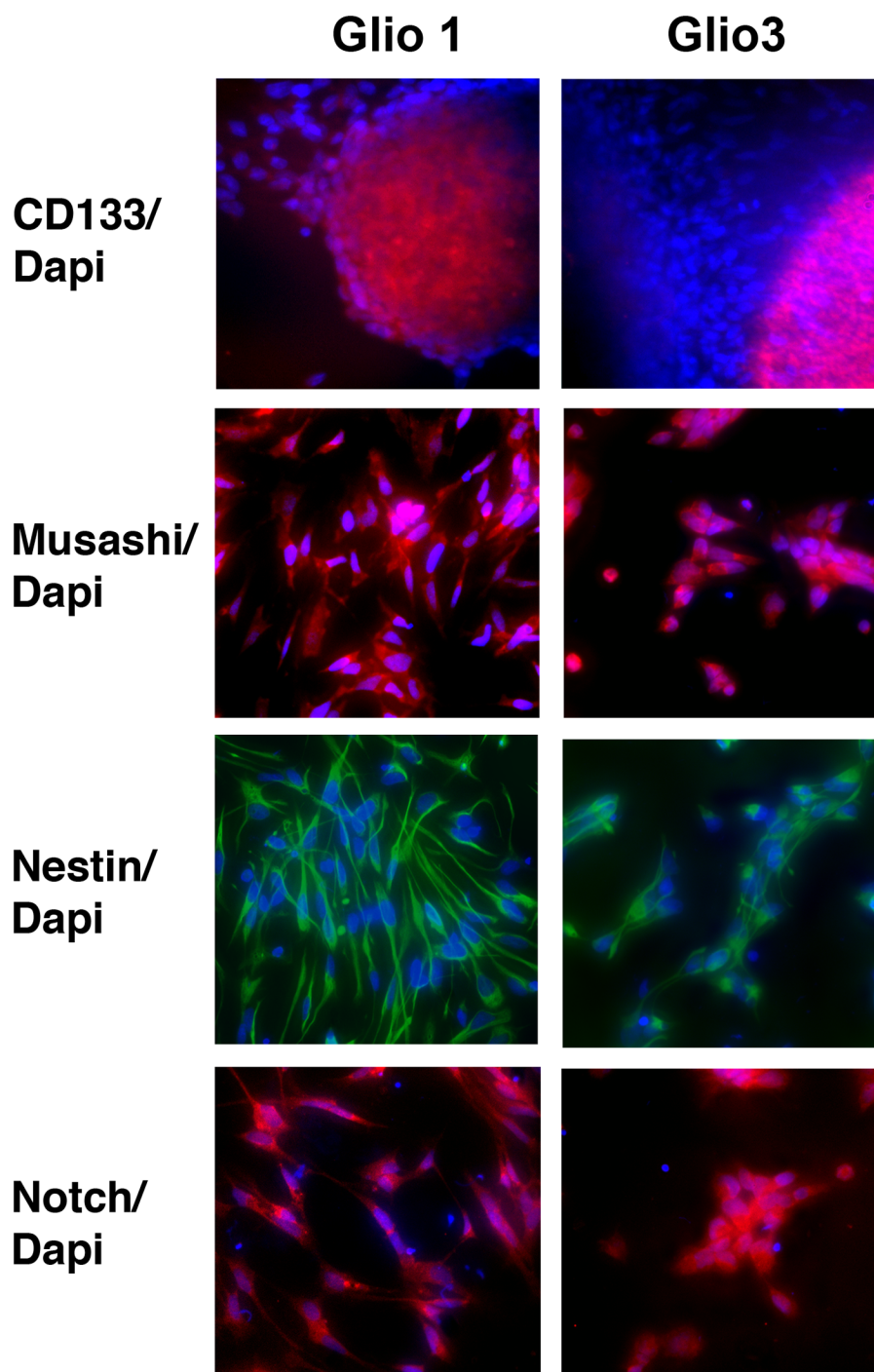
| Mean $\pm$ SEM                |           |                   |
|-------------------------------|-----------|-------------------|
| Cells                         | Compounds | IC50 ( $\mu$ M)   |
| <b>U87MG</b>                  | I-BET151  | 0.66 $\pm$ 0.014  |
|                               | TMZ       | 27.3 $\pm$ 0.48   |
|                               | BKM-120   | 1.56 $\pm$ 0.039  |
|                               | GDC0941   | 5.70 $\pm$ 1.91   |
|                               | PD0332991 | 2.41 $\pm$ 0.040  |
|                               | MLN8237   | 0.45 $\pm$ 0.27   |
|                               | MK-2206   | 1.91 $\pm$ 0.78   |
| <b>UM20 Patient Cells</b>     | I-BET151  | 0.66 $\pm$ 0.17   |
|                               | TMZ       | 137.1 $\pm$ 0.020 |
| <b>Patient GBM Stem Cells</b> | I-BET151  | 1.12 $\pm$ 0.23   |
|                               | TMZ       | 33.2 $\pm$ 8.8    |
| <b>A172 cells</b>             | I-BET151  | 1.28 $\pm$ 0.23   |
| <b>SW1783</b>                 | I-BET151  | 2.68 $\pm$ 0.45   |

**Supplemental File 1, Pastori et al.**

**A.**



**B.**



## Two-way ANOVA

| Source of Variation | % of total variation | P value  |
|---------------------|----------------------|----------|
| Interaction         | 6.18                 | < 0.0001 |
| Time                | 0                    | 1        |
| CYCLE_PHASE         | 86.86                | < 0.0001 |
| Subjects (matching) | 1.0947               | < 0.0001 |

## DMSO (G1) vs i-BET151 (G1)

| HOURS | Difference | t       | P value  | Summary |
|-------|------------|---------|----------|---------|
| 1H    | 0.2101     | 0.07134 | P > 0.05 | ns      |
| 2H    | 0.5736     | 0.1947  | P > 0.05 | ns      |
| 3H    | 0.7993     | 0.2713  | P > 0.05 | ns      |
| 4H    | -1.58      | 0.5365  | P > 0.05 | ns      |
| 5H    | 0.2411     | 0.08184 | P > 0.05 | ns      |
| 6H    | -2.489     | 0.8449  | P > 0.05 | ns      |
| 7H    | -3.928     | 1.333   | P > 0.05 | ns      |
| 8H    | -2.615     | 0.8877  | P > 0.05 | ns      |
| 9H    | -0.7283    | 0.2472  | P > 0.05 | ns      |
| 10H   | -1.882     | 0.639   | P > 0.05 | ns      |
| 11H   | -2.447     | 0.8308  | P > 0.05 | ns      |
| 12H   | -5.471     | 1.857   | P > 0.05 | ns      |
| 13H   | -5.323     | 1.807   | P > 0.05 | ns      |
| 14H   | -6.774     | 2.299   | P > 0.05 | ns      |
| 15H   | -11.01     | 3.736   | P<0.01   | **      |
| 16H   | -8.507     | 2.888   | P > 0.05 | ns      |
| 17H   | -9.681     | 3.286   | P < 0.05 | *       |
| 18H   | -8.616     | 2.925   | P > 0.05 | ns      |
| 19H   | -10.24     | 3.475   | P < 0.05 | *       |
| 20H   | -11.76     | 3.992   | P<0.01   | **      |
| 21H   | -8.739     | 2.967   | P > 0.05 | ns      |
| 22H   | -8.427     | 2.861   | P > 0.05 | ns      |
| 23H   | -6.283     | 2.133   | P > 0.05 | ns      |
| 24H   | -7.227     | 2.453   | P > 0.05 | ns      |

## DMSO (S-G2-M) vs i-BET151 (S-G2-M)

| HOURS | Difference | t      | P value  | Summary |
|-------|------------|--------|----------|---------|
| 1H    | -0.6837    | 0.2321 | P > 0.05 | ns      |

|     |        |       |          |    |
|-----|--------|-------|----------|----|
| 2H  | -6.085 | 2.066 | P > 0.05 | ns |
| 3H  | -4.611 | 1.565 | P > 0.05 | ns |
| 4H  | -6.005 | 2.038 | P > 0.05 | ns |
| 5H  | -5.864 | 1.991 | P > 0.05 | ns |
| 6H  | -7.197 | 2.443 | P > 0.05 | ns |
| 7H  | -6.858 | 2.328 | P > 0.05 | ns |
| 8H  | -7.764 | 2.636 | P > 0.05 | ns |
| 9H  | -9.186 | 3.118 | P < 0.05 | *  |
| 10H | -6.335 | 2.151 | P > 0.05 | ns |
| 11H | -5.993 | 2.034 | P > 0.05 | ns |
| 12H | -6.359 | 2.159 | P > 0.05 | ns |
| 13H | -8.909 | 3.024 | P > 0.05 | ns |
| 14H | -6.288 | 2.135 | P > 0.05 | ns |
| 15H | -4.477 | 1.52  | P > 0.05 | ns |
| 16H | -7.033 | 2.388 | P > 0.05 | ns |
| 17H | -6.017 | 2.043 | P > 0.05 | ns |
| 18H | -7.855 | 2.666 | P > 0.05 | ns |
| 19H | -6.103 | 2.072 | P > 0.05 | ns |
| 20H | -5.423 | 1.841 | P > 0.05 | ns |
| 21H | -11.19 | 3.8   | P<0.01   | ** |
| 22H | -9.355 | 3.176 | P < 0.05 | *  |
| 23H | -10.16 | 3.45  | P < 0.05 | *  |
| 24H | -8.255 | 2.802 | P > 0.05 | ns |

#### DMSO (G1-S) vs i-BET151 (G1-S)

| HOURS | Difference | t      | P value  | Summary |
|-------|------------|--------|----------|---------|
| 1H    | 0.4735     | 0.1607 | P > 0.05 | ns      |
| 2H    | 5.512      | 1.871  | P > 0.05 | ns      |
| 3H    | 3.811      | 1.294  | P > 0.05 | ns      |
| 4H    | 7.585      | 2.575  | P > 0.05 | ns      |
| 5H    | 5.623      | 1.909  | P > 0.05 | ns      |
| 6H    | 9.686      | 3.288  | P < 0.05 | *       |
| 7H    | 10.79      | 3.662  | P<0.01   | **      |
| 8H    | 10.38      | 3.523  | P < 0.05 | *       |
| 9H    | 9.915      | 3.366  | P < 0.05 | *       |
| 10H   | 8.217      | 2.79   | P > 0.05 | ns      |
| 11H   | 8.44       | 2.865  | P > 0.05 | ns      |
| 12H   | 11.83      | 4.016  | P<0.01   | **      |
| 13H   | 14.23      | 4.831  | P<0.001  | ***     |
| 14H   | 13.06      | 4.434  | P<0.001  | ***     |
| 15H   | 15.48      | 5.256  | P<0.001  | ***     |

|     |       |       |         |     |
|-----|-------|-------|---------|-----|
| 16H | 15.54 | 5.276 | P<0.001 | *** |
| 17H | 15.7  | 5.329 | P<0.001 | *** |
| 18H | 16.47 | 5.591 | P<0.001 | *** |
| 19H | 16.34 | 5.546 | P<0.001 | *** |
| 20H | 17.18 | 5.833 | P<0.001 | *** |
| 21H | 19.93 | 6.767 | P<0.001 | *** |
| 22H | 17.78 | 6.036 | P<0.001 | *** |
| 23H | 16.45 | 5.583 | P<0.001 | *** |
| 24H | 15.48 | 5.256 | P<0.001 | *** |

Bonferroni's Multiple Comparison post Repeated Measures ANOVA

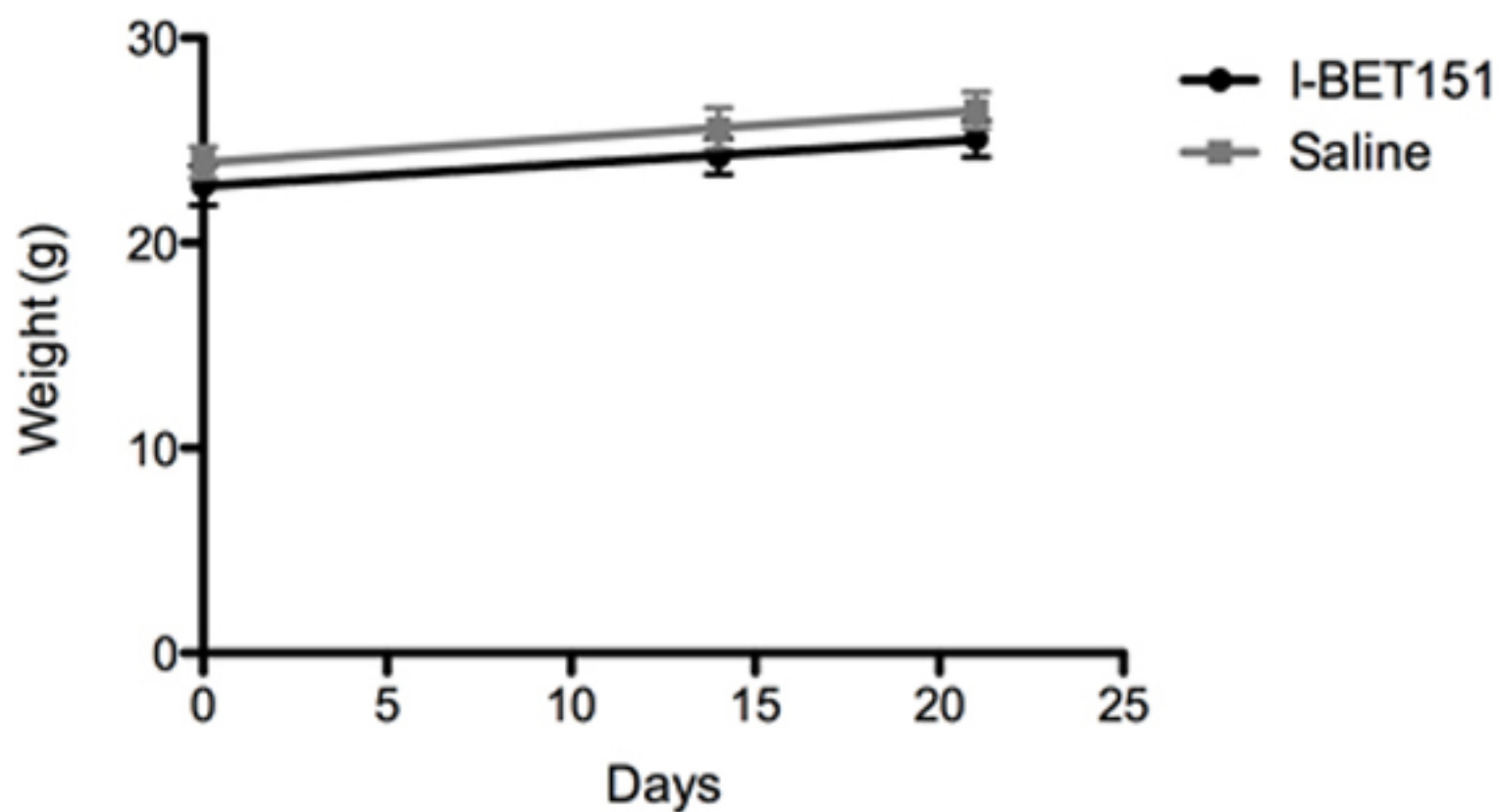
| DMSO_G1S_Fucci | Significant?<br>P < 0.05? | Summary |
|----------------|---------------------------|---------|
| 1h vs 2h       | No                        | ns      |
| 1h vs 3h       | No                        | ns      |
| 1h vs 4h       | No                        | ns      |
| 1h vs 5h       | No                        | ns      |
| 1h vs 6h       | No                        | ns      |
| 1h vs 7h       | No                        | ns      |
| 1h vs 8h       | No                        | ns      |
| 1h vs 9h       | No                        | ns      |
| 1h vs 10h      | No                        | ns      |
| 1h vs 11h      | No                        | ns      |
| 1h vs 12h      | No                        | ns      |
| 1h vs 13h      | No                        | ns      |
| 1h vs 14h      | No                        | ns      |
| 1h vs 15h      | No                        | ns      |
| 1h vs 16h      | No                        | ns      |
| 1h vs 17h      | No                        | ns      |
| 1h vs 18h      | No                        | ns      |
| 1h vs 19h      | No                        | ns      |
| 1h vs 20h      | No                        | ns      |
| 1h vs 21h      | No                        | ns      |
| 1h vs 22h      | No                        | ns      |
| 1h vs 23h      | No                        | ns      |
| 1h vs 24h      | No                        | ns      |

| I-<br>BET_G1S_Fucci | Significant?<br>P < 0.05? | Summary |
|---------------------|---------------------------|---------|
| 1h vs 2h            | No                        | ns      |

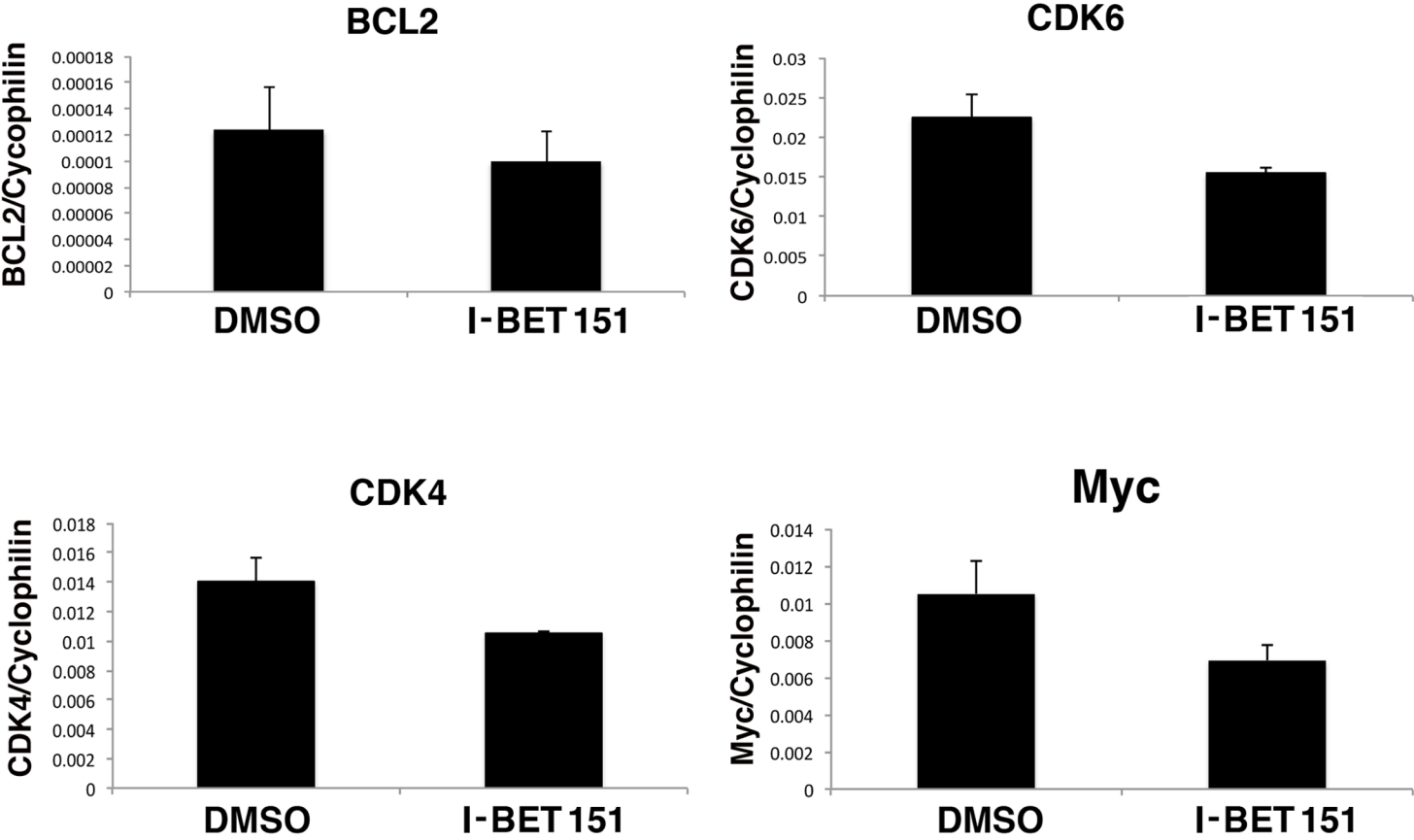
|           |     |     |
|-----------|-----|-----|
| 1h vs 3h  | No  | ns  |
| 1h vs 4h  | No  | ns  |
| 1h vs 5h  | No  | ns  |
| 1h vs 6h  | Yes | *   |
| 1h vs 7h  | Yes | *   |
| 1h vs 8h  | Yes | **  |
| 1h vs 9h  | Yes | **  |
| 1h vs 10h | Yes | **  |
| 1h vs 11h | Yes | *** |
| 1h vs 12h | Yes | *** |
| 1h vs 13h | Yes | *** |
| 1h vs 14h | Yes | *** |
| 1h vs 15h | Yes | *** |
| 1h vs 16h | Yes | *** |
| 1h vs 17h | Yes | *** |
| 1h vs 18h | Yes | *** |
| 1h vs 19h | Yes | *** |
| 1h vs 20h | Yes | *** |
| 1h vs 21h | Yes | *** |
| 1h vs 22h | Yes | *** |
| 1h vs 23h | Yes | *** |
| 1h vs 24h | Yes | *** |

**Supplemental File 3, Pastori et al.**





A.

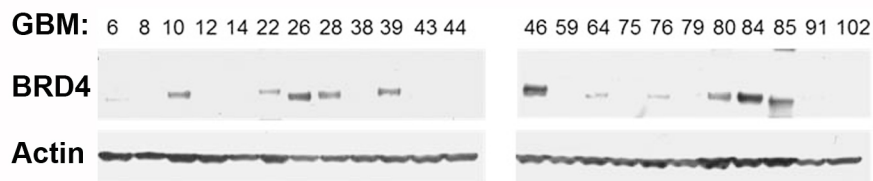


B.

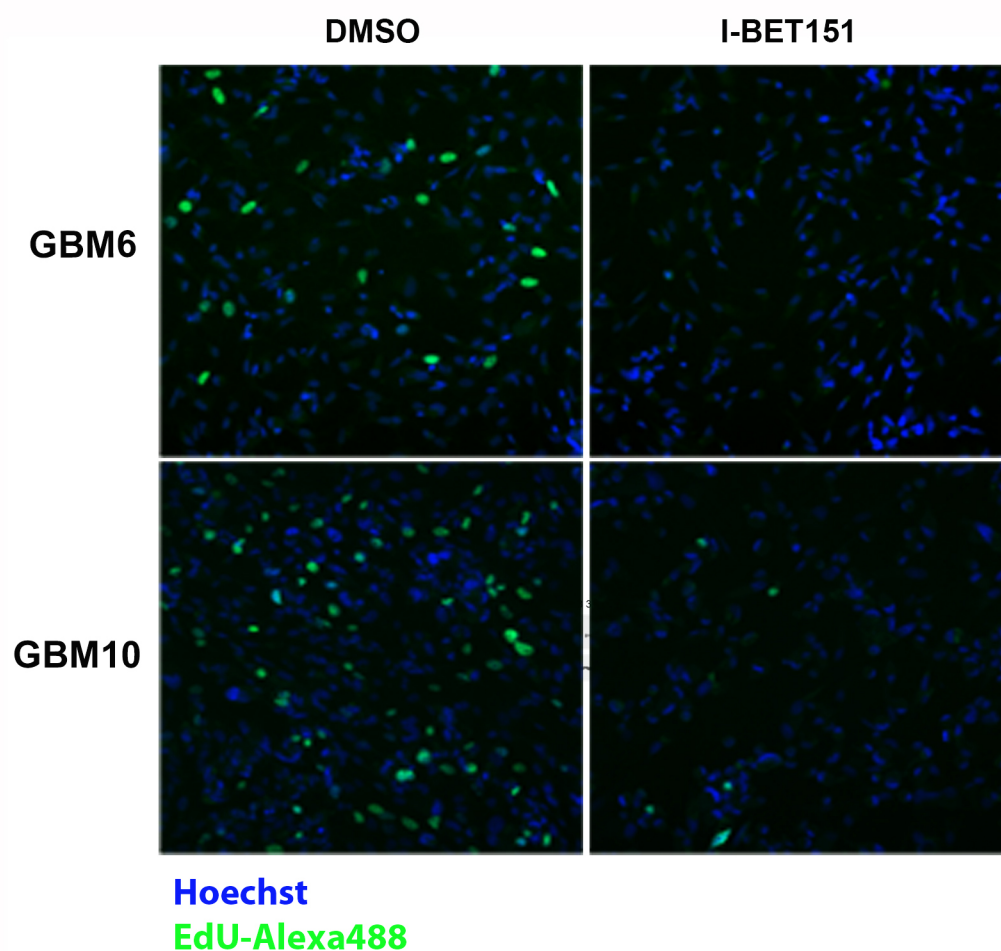
|          |   | A172 | U87  |
|----------|---|------|------|
| TLR3     | Homo sapiens toll-like receptor 3 (TLR3), mRNA.                                       | 0.30 | 0.42 |
| ---      | ncrna:misc_RNA chromosome:NCBI36:5:10390389:10390498:1 gene:ENSG00000200365           | 0.47 | 2.27 |
| ---      | Homo sapiens cDNA FLJ39257 fis, clone OCBBF2009115.                                   | 0.41 | 2.27 |
| ---      | ncrna:Mt_tRNA_pseudogene chromosome:NCBI36:9:82369444:82369510:1 gene:ENSG00000211189 | 3.62 | 2.27 |
| ---      | cdna:Genscan chromosome:NCBI36:5:14705047:14706435:-1                                 | 2.01 | 2.27 |
| ---      | Homo sapiens cDNA clone IMAGE:6380649, containing frame-shift errors.                 | 2.44 | 2.27 |
| ---      | Homo sapiens serum amyloid A2 (SAA2), mRNA  | 2.17 | 2.27 |
| HIST2H4A | Homo sapiens histone cluster 2, H4a (HIST2H4A), mRNA.                                 | 2.04 | 4.18 |

| Gene Symbol | mRna - Description   | Glio1 Fold Change | Glio3 Fold Change |
|-------------|--|-------------------|-------------------|
| CDH10       | Homo sapiens cadherin 10, type 2 (T2-cadherin) (CDH10), mRNA.  | 0.35              | 0.35              |
| TRIM22      | Homo sapiens tripartite motif-containing 22 (TRIM22), mRNA.  | 0.31              | 0.30              |
| PIPOX       | Homo sapiens pipecolic acid oxidase (PIPOX), mRNA.   | 0.46              | 0.46              |
| CAV1        | Homo sapiens caveolin 1, caveolae protein, 22kDa (CAV1), mRNA.   | 0.22              | 0.22              |
| VCAM1       | Homo sapiens vascular cell adhesion molecule 1 (VCAM1), transcript variant 1, mRNA.  | 0.46              | 0.41              |
| GPR162      | Homo sapiens G protein-coupled receptor 162 (GPR162), transcript variant A-2, mRNA.  | 0.42              | 0.41              |
| CARD8       | Homo sapiens caspase recruitment domain family, member 8 (CARD8), mRNA.  | 0.45              | 0.43              |
| HEPACAM     | Homo sapiens hepatocyte cell adhesion molecule (HEPACAM), mRNA.  | 0.30              | 0.32              |
| TMEM45A     | Homo sapiens transmembrane protein 45A (TMEM45A), mRNA.  | 0.41              | 0.43              |
| CCDC80      | Homo sapiens coiled-coil domain containing 80 (CCDC80), transcript variant 1, mRNA.  | 0.42              | 0.39              |
| LPL         | Homo sapiens lipoprotein lipase (LPL), mRNA.   |                   | 0.48              |
| IFI44L      | Homo sapiens interferon-induced protein 44-like (IFI44L), mRNA.  | 0.48              | 0.46              |
| PTX3        | Homo sapiens pentraxin-related gene, rapidly induced by IL-1 beta (PTX3), mRNA.  | 0.42              | 0.40              |
| LRRTM3      | Homo sapiens leucine rich repeat transmembrane neuronal 3 (LRRTM3), mRNA.  |                   | 0.43              |
| FIBIN       | Homo sapiens fin bud initiation factor (FIBIN), mRNA.  | 0.34              | 0.36              |
| PAK1        | Homo sapiens p21 protein (Cdc42/Rac)-activated kinase 1 (PAK1), transcript variant 1, mRNA.  | 0.43              | 0.46              |
| SCN3A       | Homo sapiens sodium channel, voltage-gated, type III, alpha subunit (SCN3A), transcript variant 1, mRNA.                           | 0.36              | 0.38              |
| PPP1R1C     | Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 1C (PPP1R1C), mRNA.   | 0.39              | 0.42              |
| LRRTM4      | Homo sapiens leucine rich repeat transmembrane neuronal 4 (LRRTM4), transcript variant 2, mRNA.                                    | 0.40              | 0.43              |
| ANKRD20B    | Homo sapiens ankyrin repeat domain 20B (ANKRD20B), non-coding RNA.   | 0.45              | 0.43              |
| FAS         | Homo sapiens Fas (TNF receptor superfamily, member 6) (FAS), transcript variant 1, mRNA.   | 0.45              | 0.41              |
| CA14        | Homo sapiens carbonic anhydrase XIV (CA14), mRNA.  | 0.45              | 0.41              |
| LRRC17      | Homo sapiens leucine rich repeat containing 17 (LRRC17), transcript variant 2, mRNA.   | 0.34              | 0.38              |
| ---         | ncrna:snoRNA_pseudogene chromosome:NCBI36:4:184487445:184487548:1 gene:ENSG00000208483   | 0.26              | 0.22              |
| PRDM1       | Homo sapiens PR domain containing 1, with ZNF domain (PRDM1), transcript variant 1, mRNA.  | 0.43              | 0.43              |
| KLRC3       | Homo sapiens killer cell lectin-like receptor subfamily C, member 3 (KLRC3), transcript variant 1, mRNA.                           | 0.23              | 0.28              |
| IFI16       | Homo sapiens interferon, gamma-inducible protein 16 (IFI16), mRNA.   | 0.40              | 0.44              |
| DENND1B     | Homo sapiens DENN/MADD domain containing 1B (DENND1B), mRNA.   | 0.41              | 0.46              |
| RAP1GAP     | Homo sapiens RAP1 GTPase activating protein (RAP1GAP), mRNA.   | 0.37              | 0.42              |
| MLC1        | Homo sapiens megalencephalic leukoencephalopathy with subcortical cysts 1 (MLC1), transcript variant 1, mRNA.                      | 0.42              | 0.41              |
| TFPI        | Homo sapiens tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor) (TFPI), transcript variant 1, mRNA.    | 0.48              | 0.42              |
| ZHX2        | Homo sapiens zinc fingers and homeoboxes 2 (ZHX2), mRNA.   | 0.46              | 0.40              |
| HOXD10      | Homo sapiens homeobox D10 (HOXD10), mRNA.  | 0.43              |                   |
| TMEM117     | Homo sapiens transmembrane protein 117 (TMEM117), mRNA.  | 0.40              | 0.46              |
| ATP13A4     | Homo sapiens ATPase type 13A4 (ATP13A4), mRNA.   | 0.36              | 0.30              |
| MAOB        | Homo sapiens monoamine oxidase B (MAOB), nuclear gene encoding mitochondrial protein, mRNA.  | 0.39              | 0.45              |
| CD200       | Homo sapiens CD200 molecule (CD200), transcript variant 2, mRNA.   | 0.27              | 0.21              |
| KLRC4       | Homo sapiens killer cell lectin-like receptor subfamily C, member 4 (KLRC4), mRNA.   | 0.24              | 0.31              |
| SEMA3E      | Homo sapiens sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E (SEMA3E), mRNA.                | 0.41              | 0.34              |
| PARP14      | Homo sapiens poly (ADP-ribose) polymerase family, member 14 (PARP14), mRNA.  | 0.29              | 0.37              |
| PION        | Homo sapiens pigeon homolog (Drosophila) (PION), mRNA.   | 0.38              | 0.45              |
| AS3MT       | Homo sapiens arsenic (+3 oxidation state) methyltransferase (AS3MT), mRNA.   | 0.41              |                   |
| ---         | Homo sapiens NKG2D mRNA, complete cds.   | 0.14              | 0.22              |
| RASGRP1     | Homo sapiens RAS guanyl releasing protein 1 (calcium and DAG-regulated) (RASGRP1), transcript variant 1, mRNA.                     | 0.40              | 0.35              |
| PDE8B       | Homo sapiens phosphodiesterase 8B (PDE8B), transcript variant 1, mRNA.   | 0.33              | 0.43              |
| RHOJ        | Homo sapiens ras homolog gene family, member J (RHOJ), mRNA.   | 0.27              | 0.36              |
| CSGALNACT1  | Homo sapiens chondroitin sulfate N-acetylgalactosaminyltransferase 1 (CSGALNACT1), transcript variant 2, mRNA.                     | 0.47              | 0.36              |
| C6orf138    | Homo sapiens chromosome 6 open reading frame 138 (C6orf138), mRNA.   | 0.46              | 0.35              |
| ELAVL4      | Homo sapiens ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D) (ELAVL4), mRNA.                            | 0.43              | 0.31              |
| ---         | Homo sapiens engulfment and cell motility 1 (ELMO1), transcript variant 2, mRNA  | 0.34              | 0.22              |
| NDP         | Homo sapiens Norrie disease (pseudoglioma) (NDP), mRNA.  | 0.36              | 0.48              |
| GABRB1      | Homo sapiens gamma-aminobutyric acid (GABA) A receptor, beta 1 (GABRB1), mRNA.   | 0.47              | 0.34              |
| ---         | ncrna:Mt_tRNA_pseudogene chromosome:NCBI36:1:179658667:179658737:1 gene:ENSG00000208272  | 0.38              | 0.22              |
| ---         | ncrna:misc_RNA chromosome:NCBI36:6:100080694:100080802:1 gene:ENSG00000207017  | 0.39              | 0.22              |
| GDF15       | Homo sapiens growth differentiation factor 15 (GDF15), mRNA.   | 0.38              | 0.22              |
| FLJ30594    | Homo sapiens cDNA FLJ34044 fis, clone FCBBF2007080.  | 0.27              | 0.47              |
| ---         | ncrna:misc_RNA chromosome:NCBI36:2:230631189:230631284:-1 gene:ENSG00000199400   | 0.42              | 0.22              |
| ---         | ncrna:snRNA chromosome:NCBI36:4:3015626:3015732:1 gene:ENSG00000199335   | 0.42              | 0.22              |
| ---         | Homo sapiens cDNA FLJ33625 fis, clone BRAMY2022052.  | 0.42              | 0.22              |
| ---         | cdna:pseudogene chromosome:NCBI36:1:203586998:203587222:1 gene:ENSG00000220805   | 0.43              | 0.22              |
| ---         | cdna:known chromosome:NCBI36:9:40297346:40329528:1 gene:ENSG00000185020  | 0.44              | 0.22              |
| ---         | cdna:pseudogene chromosome:NCBI36:7:151694985:151695278:1 gene:ENSG00000204894   | 0.45              | 0.22              |
| ---         | Homo sapiens protocadherin alpha 7 (PCDHA7), transcript variant 2, mRNA  | 0.48              | 0.22              |
| ---         | ncrna:snRNA chromosome:NCBI36:1:142478882:142479043:1 gene:ENSG00000206694   | 0.48              | 0.22              |
| ---         | Homo sapiens potassium large conductance calcium-activated channel, subfamily M beta member 3 (KCNMB3), transcript variant 2, mRNA |                   | 0.22              |
| SCRG1       | Homo sapiens scrapie responsive protein 1 (SCRG1), mRNA.   | 0.19              | 0.43              |
| DCXR        | Homo sapiens dicarbonyl/L-xylulose reductase (DCXR), mRNA.   | 2.43              | 2.14              |
| HIST2H4A    | Homo sapiens histone cluster 2, H4a (HIST2H4A), mRNA.  | 3.50              | 3.14              |
| HIST1H2BD   | Homo sapiens histone cluster 1, H2bd (HIST1H2BD), transcript variant 1, mRNA.  | 3.63              | 2.23              |
| HIST2H2BF   | Homo sapiens histone cluster 2, H2bf (HIST2H2BF), mRNA.  | 3.52              | 2.06              |
| HIST2H2BE   | Homo sapiens histone cluster 2, H2be (HIST2H2BE), mRNA.  | 3.67              | 2.09              |
| ---         | ncrna:RNA chromosome:NCBI36:6:162211091:162211197:-1 gene:ENSG00000222199  | 2.11              | 0.22              |
| ---         | ncrna:tRNA_pseudogene chromosome:NCBI36:1:146292313:146292384:1 gene:ENSG00000208836   | 2.11              | 0.22              |
| ---         | ncrna:tRNA_pseudogene chromosome:NCBI36:11:67984192:67984263:-1 gene:ENSG00000210241   | 2.22              | 0.22              |
| HSPA6       | Homo sapiens heat shock 70kDa protein 6 (HSP70B') (HSPA6), mRNA.   | 2.39              | 0.29              |
| ---         | ncrna:snRNA chromosome:NCBI36:1:142438537:142438700:-1 gene:ENSG00000207216  | 2.36              | 0.22              |
| ---         | Homo sapiens cDNA clone IMAGE:6380649, containing frame-shift errors.  | 2.52              | 0.22              |
| ---         | ncrna:tRNA_pseudogene chromosome:NCBI36:6:28709838:28709909:-1 gene:ENSG00000210508  | 2.99              | 0.22              |

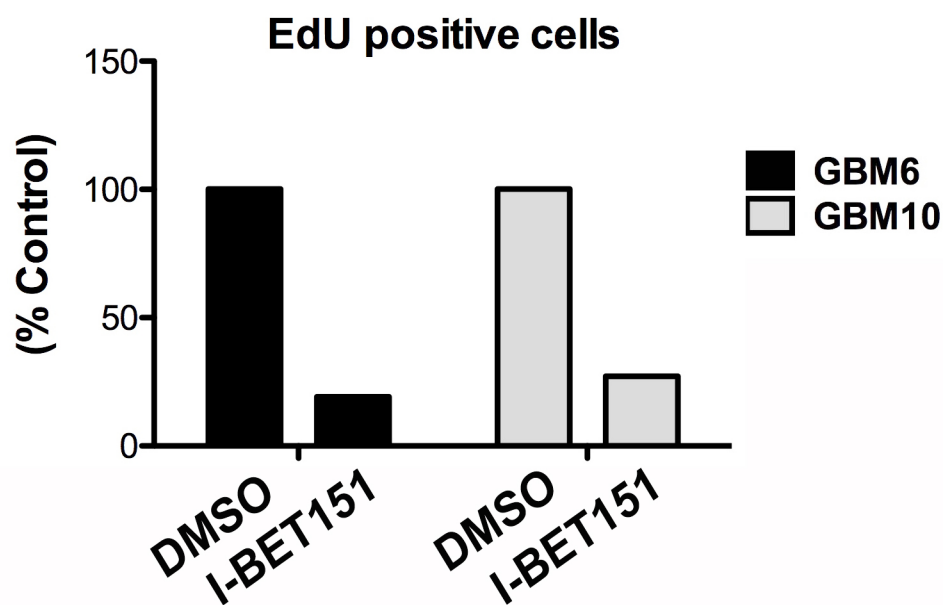
## Supplemental File 6, Pastori et al



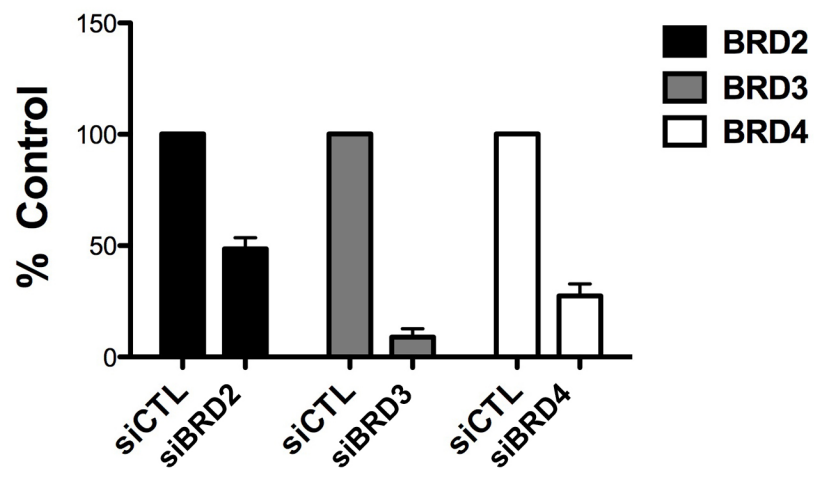
**B.**



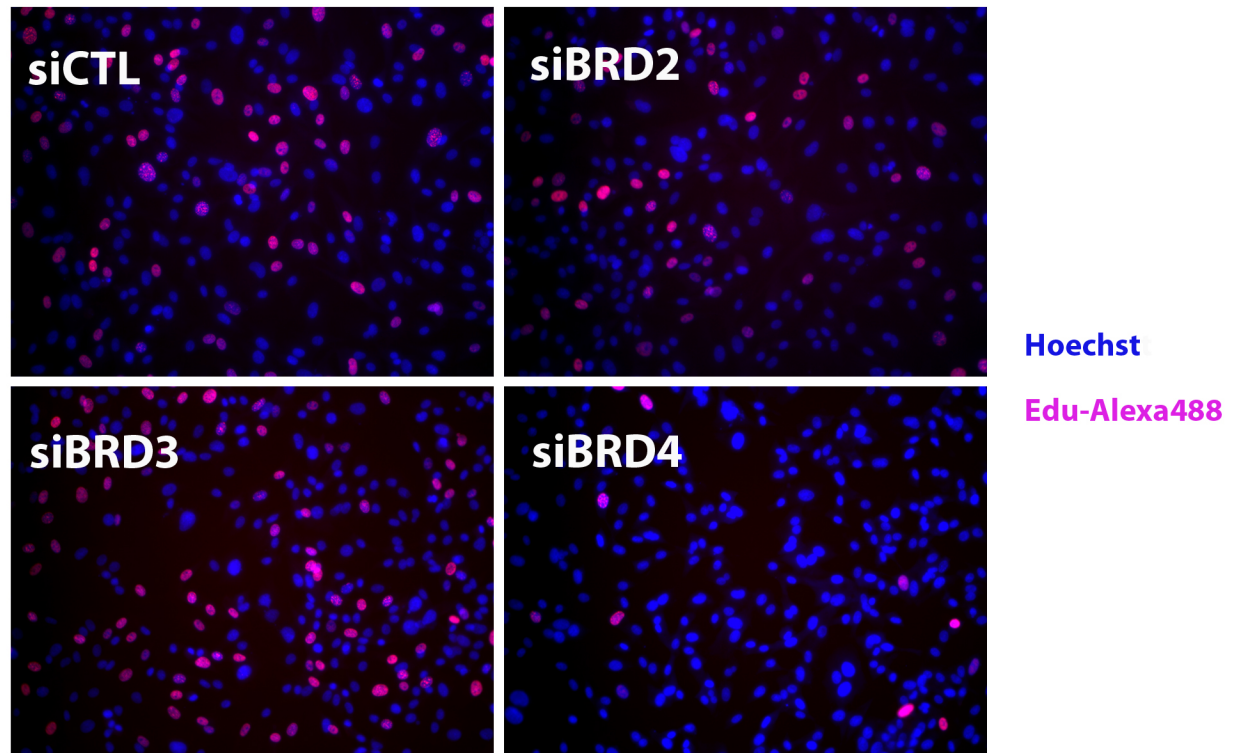
**C.**



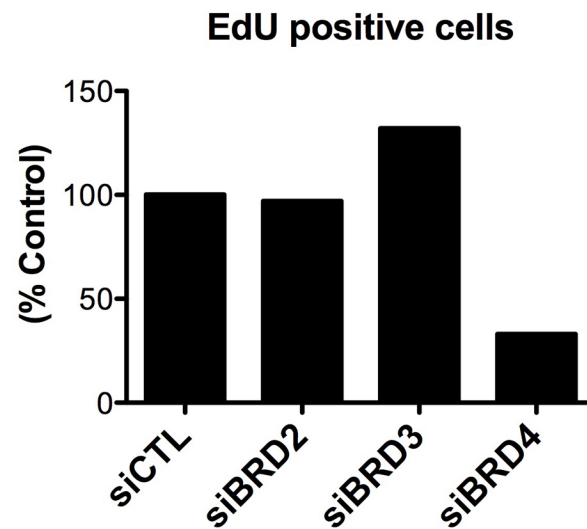
**A.**



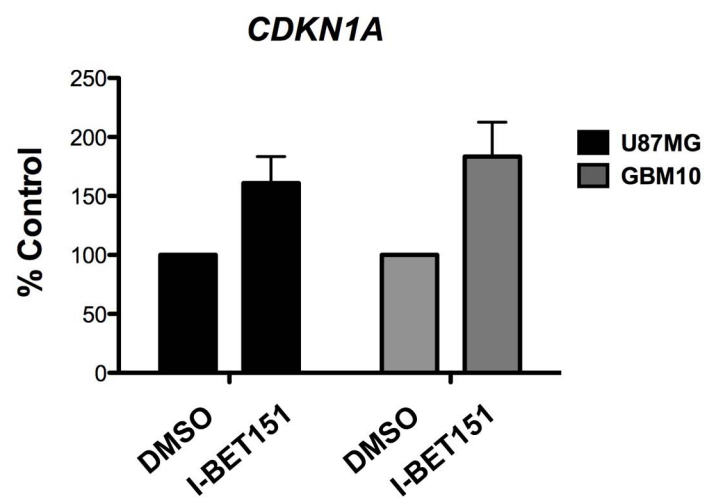
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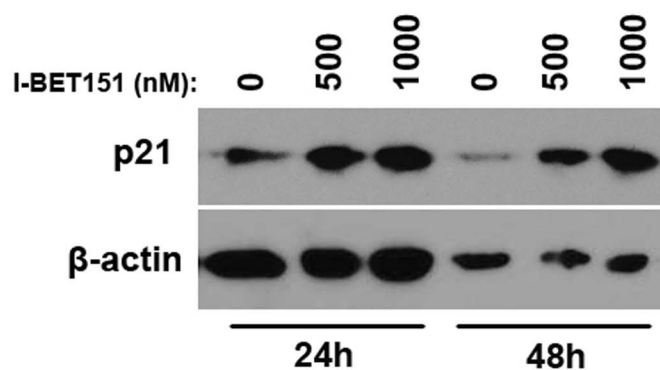
**C.**



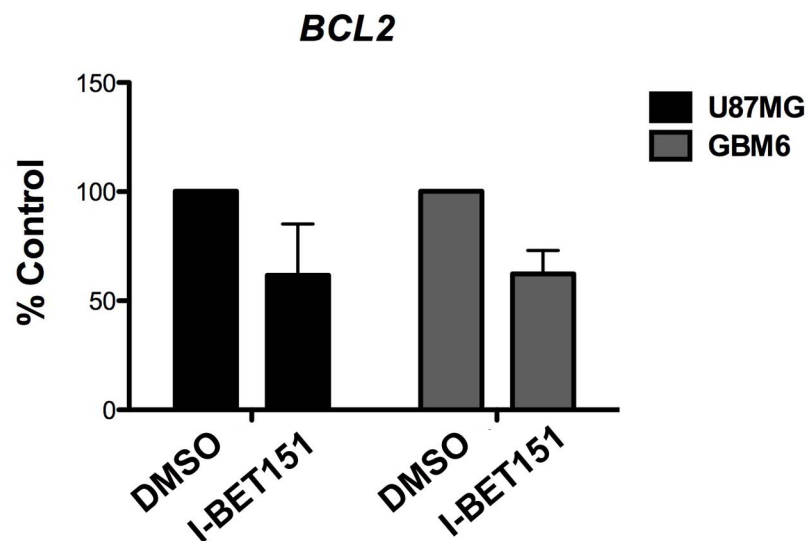
A.



B.



C.



D.

